



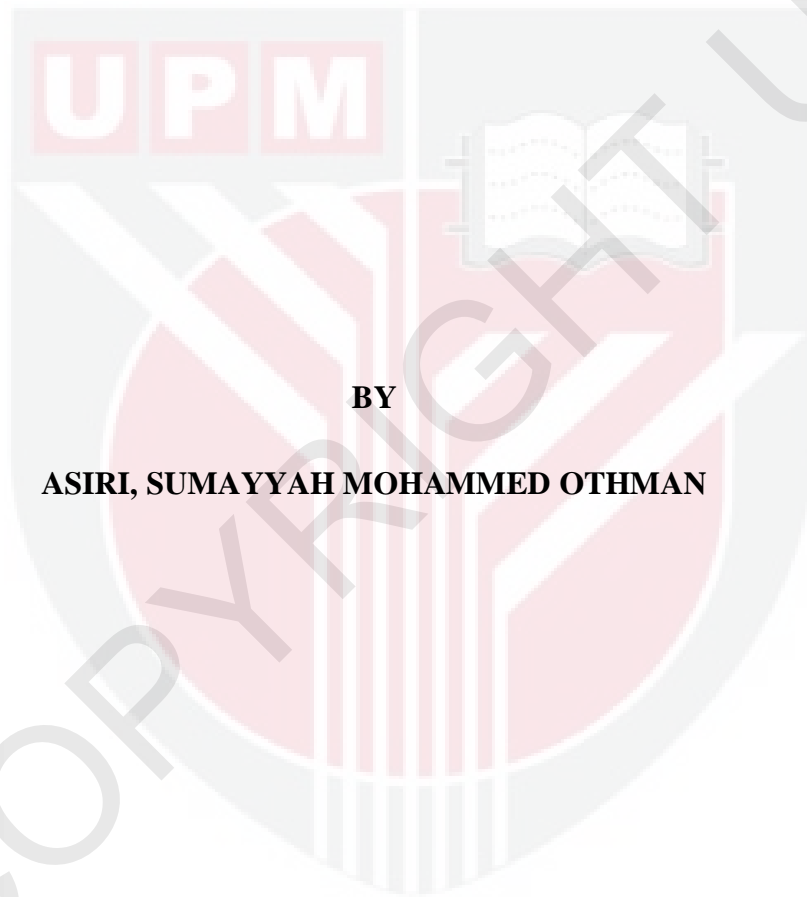
**UNIVERSITI PUTRA MALAYSIA**

**PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF *CALLICARPA*  
*MAINGAYI* K. & G.**

**ASIRI, SUMAYYAH MOHAMMED OTHMAN**

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**BY**

**ASIRI, SUMAYYAH MOHAMMED OTHMAN**

**MASTER OF SCIENCE  
UNIVERSITI PUTRA MALAYSIA**

**2012**

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**ASIRI, SUMAYYAH MOHAMMED OTHMAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfillment of the Requirements for the Degree of Master of Science**

**February 2012**

## DEDICATION

*I dedicate this thesis to:*

*My father, Mohammed Asiri, who taught me that the best kind of knowledge to have is that which is learned for its own sake.*

*My mother, Hajjar Alaki, who taught me that even the largest task can be accomplished if it is done one step at a time.*

*My lovely brothers and sisters, whose love and devotion provided me motivation*

*My friends, whose always have place in my heart*

*To all those who believe in the richness of learning.*

*Sumayyah Asiri*

Abstract of thesis presented to the Senate of University Putra Malaysia in  
Fulfillment of the requirement for the degree of Master of Science

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By

**ASIRI, SUMAYYAH MOHAMMED OTHMAN**

**February 2012**

**Chairman: Professor Md. Nordin Hj. Lajis, PhD**

**Institute: Bioscience**

*Callicarpa maingayi*, which is known in Malaysia as “tampang besi” belongs to Lamiaceae family. The plants of the genus *Callicarpa* have been used to treat various ailments, such as malaria, skin cancer, indigestion, dropsy, stomach disorders and rheumatism.

The stem bark of *Callicarpa maingayi* was exhaustively extracted using methanol-water (8:2). The crude extract was successively fractionated using *n*-hexane, chloroform and ethyl acetate. Separation, isolation and purification of compounds were done using solvent-solvent partitioning and chromatographic techniques such as High Performance Liquid chromatography (HPLC), as well as normal phase, reverse phase and Sephadex LH-20 column chromatography.

From the hexane extract, three known compounds were isolated, namely palmitic acid (**91**), tetracosanoic acid (**92**) and a mixture of stigmasterol and  $\beta$ -sitosterol (**93**).

The investigation on the chloroform extract led to the isolation of two new naphthoquinone, (+)-callicarpaquinone-A (**97**), callicarpaquinone-B (**96**), along with known avicequinone-C (**95**), which was isolated from the first time from *Callicarpa* species, and  $3\beta$ -hydroxy-lup-20(29)-en-28-oic-acid (**94**).

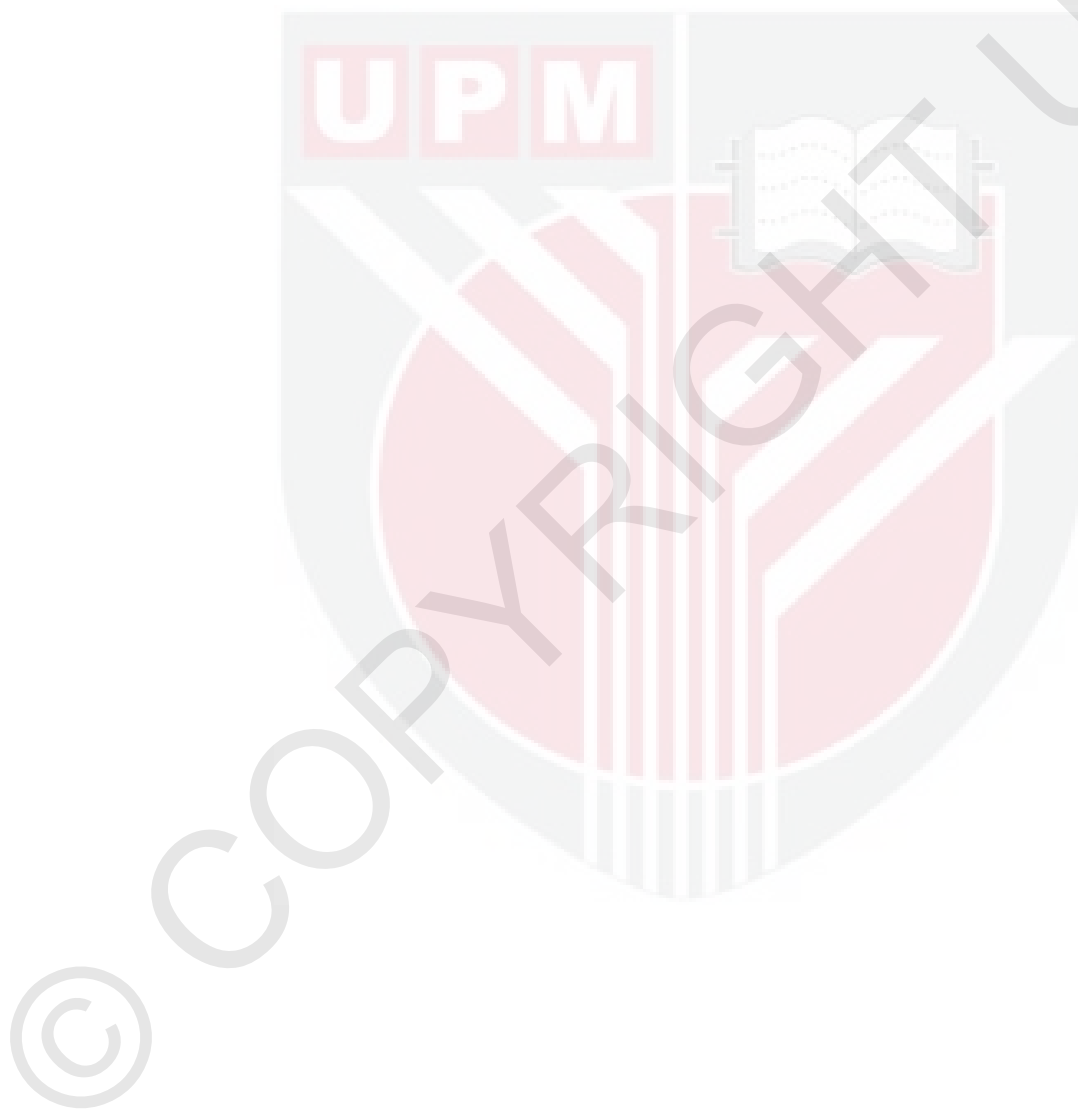
From the ethyl acetate extract, three known compounds (+)-paulownin (**98**), (-)-wodeshiol (**99**), which were isolated for the first time from *Callicarpa* species together with  $\beta$ -sitosterol- $\beta$ -D-glucopyranoside (**100**).

The extracts and isolated compounds were screened *in vitro* for anticholinesterase and cytotoxic activities using Ellman's and MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) methods.

The hexane, chloroform and ethyl acetate extracts were inactive against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with  $IC_{50}$  values of  $> 100 \mu\text{M}$ , but were found to exhibit significant cytotoxicity against liver cancer (HepG2) and breast cancer (MCF-7) cell lines with  $IC_{50}$  values ranging from 12.5 to 25.0  $\mu\text{g/mL}$ . The isolated compounds, (+)-callicarpaquinone-A (**97**), callicarpaquinone-B (**96**), avicequinone-C (**95**), (+)-paulownin (**98**) and (-)-wodeshiol (**99**) were inactive against

AChE with  $IC_{50}$  values of  $> 100 \mu\text{M}$ . However, they were active against the MCF-7 breast cancer cell line with  $IC_{50}$  values of 25.0, 1.9, 2.3, 14.0 and  $14.0 \mu\text{M}$ , respectively.

The structures of the isolated compounds were elucidated using spectroscopic techniques including UV, IR, MS,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, DEPT, HMBC and  $^1\text{H}$ - $^1\text{H}$  COSY.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KAJIAN FITOKIMIA DAN BIOAKTIVITI *CALLICARPA MAINGAYI* K. & G.**

Oleh

**ASIRI, SUMAYYAH MOHAMMED OTHMAN**

**Februari 2012**

**Pengerusi: Profesor Md. Nordin Hj. Lajis, PhD**

**Institut: Biosains**

*Callicarpa maingayi*, dikenali di Malaysia sebagai "tampang Besi" adalah dari keluarga Lamiaceae. Tumbuhan dari genus *Callicarpa* telah digunakan untuk merawat pelbagai penyakit seperti malaria, kanser kulit, senak, dropsi, gangguan perut dan reumatisme

Kulit batang *Callicarpa maingayi* diekstrak menggunakan pelarut metanol air (8:2). Ekstrak mentah diperingkat menggunakan pelarut heksana, kloroform dan etil asetat. Pemisahan, pengasingan dan penulenan sebatian telah dilakukan dengan menggunakan pemeringkatan pelarut dan teknik kromatografi seperti kromatografi cecair prestasi tinggi (HPLC), serta kromatografi fasa biasa, fasa terbalik dan Sephadex LH-20.

Dari ekstrak heksana tiga sebatian yang dikenali telah berjaya dipencilkan iaitu asid palmitik (**91**), asid tetrakosanoik (**92**) dan campuran stigmasterol dan  $\beta$ -sitosterol (**93**).

Kajian terhadap ekstrak kloroform telah berjaya mengasingkan dua sebatian naphthokuinon baharu iaitu, callicarpaquinone-A (97), callicarpaquinone-B (96), bersama-sama dengan avicequinone-C (95), yang telah diasingkan buat kali pertama dari spesies *Callicarpa* dan asid 3 $\beta$ -hydroxy-lup-20 (29)-en-28oik (94).

Dari ekstrak etil asetat 3 sebatian yang dikenali iaitu paulownin (98), wodeshiol (99), berjaya dipencilkan untuk kali yang pertama dari spesies *Callicarpa* bersama-sama dengan  $\beta$ -sitosterol- $\beta$ -D-glucoopyranoside (100).

Ekstrak mentah dan sebatian terpencil telah diuji untuk aktiviti antikolinesterase dan sitotoksik secara *in vitro* menggunakan kaedah Ellman dan MTT (3-(4,5-dimethylthiazole-2-yl) -2,5-diphenyltetrazolium bromide).

Ekstrak pelarut heksana, kloroform dan etil asetat didapati tidak aktif terhadap aktiviti antiasetilkinesterase (AChE) dan antibutirilkolinesterase (BChE) dengan nilai IC<sub>50</sub> >100  $\mu$ M tetapi telah menunjukkan aktiviti sitotoksik yang ketara terhadap sel kanser hati (HepG2) dan sel kanser payudara (MCF-7) dengan nilai IC<sub>50</sub> antara 12.5 kepada 25.0  $\mu$ g/mL. Sebatian yang berjaya dipencilkan callicarpaquinone-A (97), callicarpaquinone-B (96), avicequinone-C (95), paulownin (98) dan wodeshiol (99) tidak aktif terhadap aktiviti antiasetilkinesterase dengan nilai IC<sub>50</sub> > 100  $\mu$ M. Tetapi aktif terhadap sel MCF-7 dengan nilai IC<sub>50</sub> masing-masing sebanyak 25.0, 1.9, 2.3, 14.0 dan 14.0  $\mu$ M.

Struktur sebatian yang berjaya dipencilkan telah dikenalpasti menggunakan teknik spektroskopi termasuk UV, IR, MS,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, DEPT, HMBC and  $^1\text{H}$ - $^1\text{H}$  COSY.



## **ACKNOWLEDGEMENT**

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To each of the above, I extended my deepest appreciations.

**SUMAYYAH MOHAMMED OTHMAN ASIRI**

I certify that an Examination Committee has met on 20/2/212 to conduct the final examination of Asiri, Sumayyah Mohammed on her Master of Science thesis entitled “PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF *CALLICARPA MAINGAYI*” in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**MOHD ASPOLLAH HJ SUKARI, PhD**

Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**GWENDOLINE CHENG LIAN EE, PhD**

Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Internal Examiner)

**INTAN SAFINAR ISMAIL, PhD**

Doctor  
Faculty of Science  
Universiti Putra Malaysia  
(Internal Examiner)

**JALIFAH BINTI LATIP, PhD**

Associate Professor  
Faculty of Science and Technology  
Universiti Kebangsaan Malaysia  
(External Examiner)

---

**Prof. Dr. Bujang Kim Huat, PhD**

Professor/ Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement of the degree of Master of Science. The members of the supervisory Committee were as follows:

**Mohd. Nordin Hj. Lajis, PhD**

Professor  
Institute of Bioscience  
Universiti Putra Malaysia  
(Chairman)

**Khozirah Shaari, PhD**

Professor  
Institute of Bioscience  
Universiti Putra Malaysia  
(Member)

**Faridah Abas**

Associate Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Member)

---

**BUJANG KIM HUAT, PHD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia  
Date:

## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

---

**ASIRI, SUMAYYAH MOHAMMED OTHMAN**

Date: 20 February 2012



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